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CALORIMETRIC INVESTIGATIONS ON SYSTEMS
OF BIOCHEMICAL INTEREST WITH PARTICULAR
EMPHASIS ON HEAT CAPACITY MEASUREMENTS

Lund 1973

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by

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Printed in Sweden
Studentlitteratur
Lund 1973

This review gives the summary of results from the following papers which will be referred to by Roman numerals I-V.

- I. Konicek, J., Suurkuusk, J. and Wadsö, I.
A Precise Drop Heat Capacity Calorimeter for Small Samples.
Chemica Scripta 1 (1971) 217.
- II. Suurkuusk, J. and Wadsö, I.
Design and Testing of an Improved Precise Drop Heat Capacity Calorimeter for Small Samples.
J. Chem. Thermodynamics. In press.
- III. Kusano, K., Suurkuusk, J. and Wadsö, I.
Thermochemistry of Solutions of Biochemical Model Compounds.
2. Alkoxyethanols and 1,2-Dialkoxyethanes in Water.
J. Chem. Thermodynamics 5 (1973) 757.
- IV. Suurkuusk, J.
Specific Heat Measurements on Lysozyme, Chymotrypsinogen and Ovalbumin in Aqueous Solution and in Solid State.
Acta Chem. Scand. In press.
- V. Suurkuusk, J. and Wadsö, I.
Thermochemistry of the Avidin-Biotin Reaction.
Eur. J. Biochem. 28 (1972) 438.



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INTRODUCTION

The solvation of non-polar molecules in water is accompanied mostly by a favourable enthalpy change, which can not wholly be accounted for by the consideration of differences in the non-bonding interactions between solute-solute and solute-water molecules. The first characterization of this phenomenon was proposed, by Frank and Evans (1), to be due to the ability of non-polar species to increase the hydrogen bonding in water, which causes the observed enthalpy decrease upon solvation. This 'structuring' of water in the proximity of non-polar molecules decreases the entropy to such an extent that the exothermic enthalpy is more than compensated for and the free energy becomes positive. This is reflected as a low solubility of non-polar molecules in water. The reverse of the solvation process is the formation of hydrophobic bonds, where ΔS is the driving force. Kauzmann (2) pointed out the above properties of water to be one of the most important factors for the stabilization of the three-dimensional structure of proteins, due to the favourable entropy in forming hydrophobic bonds between non-polar side groups in proteins. The numerical treatment of the stabilization contributions from non-polar species in a protein, which was developed by Nemethy and Scheraga (3,4) by use of the "flickering clusters" model of water proposed by Frank and Wen (5), seems to be the most widely accepted model for statistical calculations. Although this model can account for many of the thermodynamic properties, it seems to fail in the prediction of the heat capacities. The abnormal apparent heat capacity first observed by Edsall (6) for non-polar molecules in water, is caused by the more rapid continuous "melting" of water structure with increasing temperature in a non-polar aqueous solution than in pure water (7).

There are several different models beside that offered by Nemethy-Scheraga which fact indicates the complexity of these problems, and that our understanding of the properties of water is far from clear.

The main aim of the work discussed in the present summary has been to obtain basic thermodynamic information, which will contribute to the

understanding of the interactions of water with proteins and the role of water in biological systems.

Paper III is part of a systematic calorimetric investigation on the properties of solutions of simple organic compounds, which may be looked upon as biochemical models. The structures of the model compounds (III, ref 8) have been varied systematically and, from derived thermodynamic parameters, it has been possible to correlate structural features with the solute-water interactions. In paper III enthalpies of solution, ΔH_{soln} , were obtained at three different temperatures for a number of ethylene glycol derivatives, $\text{ROCH}_2\text{-CH}_2\text{OH}$ and $\text{ROCH}_2\text{-CH}_2\text{OR}'$, where R and R' are alkyl groups. The results from the enthalpy of solution measurements were used to calculate the excess heat capacity ΔC_p^0 for the process, and by combining these results with the heat capacity, C_p^0 , for the pure compounds the partial heat capacities, $\bar{C}_{p,2}^0$, in aqueous solution were obtained. The C_p^0 values were measured by the drop heat capacity calorimeter described in paper I.

The above method for deriving $\bar{C}_{p,2}^0$ values in aqueous solution is not applicable to compounds for which solution processes are slow, and for compounds like proteins which are usually available only in small quantities. Paper II describes the design and testing of an improved version of the earlier (I) drop heat capacity calorimeter, which allows direct determination of solute heat capacities in dilute solutions. The precision of such measurements must be very high, approaching 0.01 per cent, in order to obtain $\bar{C}_{p,2}^0$ values with errors less than a few per cent for dilute (~ 0.05 M) aqueous solutions.

The results from the model compound experiments (III, ref 8) show that the excess heat capacities, when hydrophobic molecules are solvated by water, can be accounted for by simple additivity rules. From these results and from the obtained heat capacity values for several proteins in the solid state as well as in aqueous solution (paper IV) it was concluded that the large ΔC_p for transferring a protein to aqueous solution is caused by the hydration of a substantial number of non-polar side groups. The analysis of the heat capa-

city values for proteins in aqueous solution gives information about the distribution of non-polar groups between the interior and the surface of a protein.

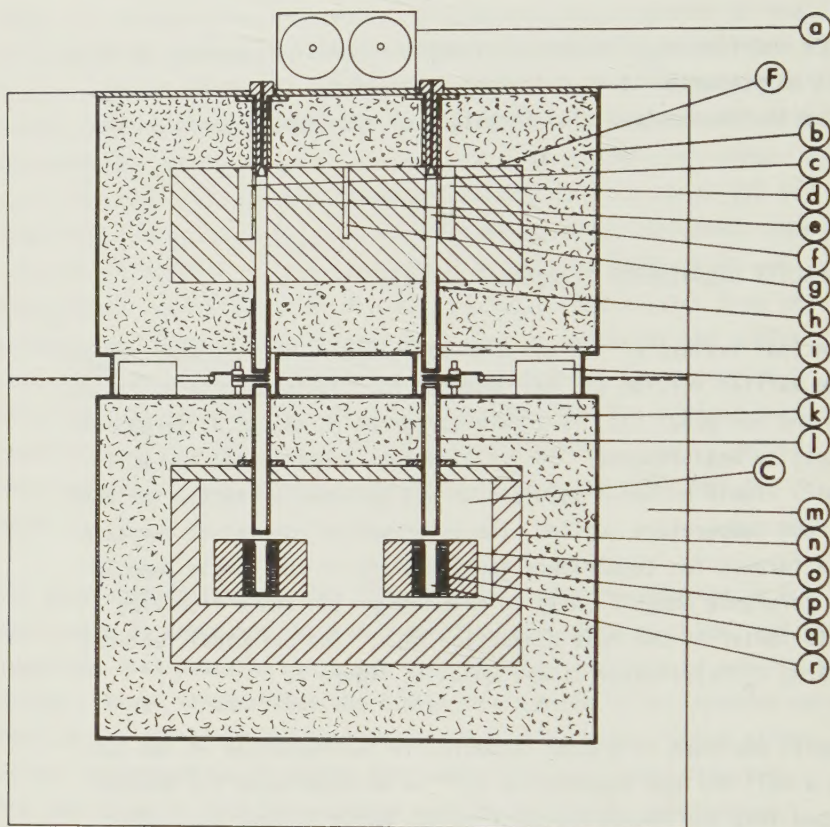
The large negative ΔC_p for the avidin-biotin reaction obtained by heat of reaction measurements at different temperatures (paper V) is consistent with the proposed (16) formation of hydrophobic bonds in the process.

HEAT CAPACITY CALORIMETER (Papers I and II)

The principal feature of the two drop calorimeters is the same, except that the earlier version (I) was designed for single ampoule operation whereas the new model (II) is a twin design which allows differential heat capacity measurements. The calorimeters are intended for work with small liquid or solid samples and are designed primarily for work in the room temperature region. The calorimeters consist of two main parts: a furnace for temperature equilibration of the sample ampoule and the reference ampoule (only in calorimeter II) and a receiving twin calorimeter of the heat conduction type. The principal features of the drop C_p calorimeter II are shown in figure 1.

The sample, enclosed in a steel ampoule, is thermostatted in the furnace at a well defined temperature θ_i . In an experiment the ampoule is dropped into the receiving calorimeter, which is kept at another well-defined temperature θ_f . The twin calorimeter measures the differences in heat contents $H_f - H_i$ between the two temperatures θ_f and θ_i . From the difference obtained with the sample ampoule filled and empty, and from the measured temperature difference between the furnace and calorimeter ($\theta_f - \theta_i$) the mean heat capacity for the temperature interval $\theta_f - \theta_i$ can be calculated. In calorimeter II, when measuring C_p for a solution, the contributions from the solvent can be cancelled out if the reference ampoule is filled with an equal amount of pure solvent.

Figure 1. Schematic view of the calorimetric assembly.



F, furnace; C, twin calorimeter; a, mechanical lift; b, insulation; c, hole; d, hole for the quartz probe; e, f, holes for equilibration of the ampoules; g, hole for the thermistor; h, copper tube; i, plastic tube; j, electromechanical shutter; k, insulation; l, perforated plastic tube; m, water thermostat; n, main heat sink; o, calorimetric unit; p, aluminium block; q, thermocouple plate; r, receiver for the ampoules.

The calibration of the calorimeters may be determined by absolute measurements i.e. by electrical calibration, or by relative calibration using water as calibration standard, as its heat capacity has been very carefully determined at National Bureau of Standards (NBS), Washington, D.C. With electrical calibration a small but significant systematic error is obtained for calorimeter II. The most probable cause for the slightly higher calibration constants obtained is heat loss through the heater leads. The relative calibration constant was used in heat capacity determinations with calorimeter II, whereby a number of possible systematic errors are cancelled or avoided.

Experiments performed on test substances, for which C_p values have been determined at NBS are summarized in Table 1.

Table 1. Results of experiments with test substances

Compound	$C_p^0(25^\circ\text{C})/\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$		
	Calorimeter I ^a	Calorimeter II ^b	Lit.
Water	75.35 ± 0.03	—	75.30 ± 0.01 Ref 9
Sapphire	79.16 ± 0.03	79.15 ± 0.02	79.01 ± 0.08 Ref 10
Benzoic acid	146.8 ± 0.2	146.78 ± 0.06	146.8 ± 0.3 Ref 11
Heptane	—	224.73 ± 0.05	224.7 ± 0.2 Ref 12

^aelectrical calibration

^bcalibration with water

Each C_p value is a weighted mean value obtained from several test series, where each series consisted of 4-8 experiments. The sample quantity is in the 0.5-1.0 gram range. The errors given are twice the standard deviation of the mean. The values in table 1 are in full agreement with the NBS-values in the last column except for sapphire.

For sapphire the present value is about 0.2 per cent higher than the NBS-value, which indicates a small systematic difference. Calorimeter II was also tested by the direct determination of $\bar{C}_{p,2}^0$ for diethoxyethane in dilute aqueous solution (0.04-0.06 M). The obtained value, $\bar{C}_{p,2} = 571 \pm 7 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$ is in full agreement with the value $\bar{C}_{p,2}^0 = 569 \pm 7 \text{ J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$ (paper III), which was obtained through the indirect method (eqn 2). From the results of the test experiments the absolute accuracy is believed to be better than 0.1 per cent for both calorimeters. The precision of the C_p determinations are found to be in the order of 0.05 per cent and 0.01 per cent for the C_p calorimeters I and II, respectively, using 1 gram samples and a temperature difference of 10°C .

The improvement of the precision and reliability of the new model compared with the other model is largely ascribed to the improved electronic equipment and improved mechanical design. Under the experimental conditions reported, the differential principle has not been shown to improve the properties over that of a corresponding single ampoule operation.

THERMOCHEMICAL PROPERTIES OF ALKOXYETHANOLS AND 1,2-DIALKOXYETHANES IN WATER (Paper III)

The enthalpy of solution measurements were performed on an LKB-8721-1 Precision Calorimeter at 20, 25 and 30°C on ethylene glycol derivatives $\text{ROCH}_2\text{-CH}_2\text{-OH}$ and $\text{ROCH}_2\text{-CH}_2\text{-OR}'$, where R and R' are methyl-, ethyl-, propyl-, i-propyl- and butylgroups. The final concentrations were in the range 0.01-0.05 M. The ΔH_{soln}^0 values were obtained by linear extrapolation to zero concentration. The enthalpy of solvation ΔH_{solv}^0 was calculated by use of eqn 1,

$$\Delta H_{\text{solv}}^0 = \Delta H_{\text{soln}}^0 - \Delta H_v^0, \quad (1)$$

and the enthalpy of vaporisation ΔH_v^0 values for the compounds are obtained from ref 13 and 14. The heat capacity change accompanying the solution process, $\Delta C_{p,2}^0$, was calculated from the temperature depend-

ency of ΔH_{soln} . Combining the $\Delta C_{p,2}^0$ values with the measured C_p^0 values for the pure compounds according to eqn 2,

$$\bar{C}_{p,2}^0 = C_p^0 + \Delta C_p, \quad (2)$$

the partial heat capacity at infinite dilution, $\bar{C}_{p,2}^0$, is obtained. The results are summarized in table 2.

In figure 2 values for $\Delta H_{\text{soln}}(25^\circ\text{C})$ are plotted against the total number of carbon atoms, n . It is seen that the minimum value in ΔH_{soln}^0 occurs at a different number of carbon atoms for the straight chain monoethers and for the symmetrical and asymmetrical diethers. The same pattern has also been found for other compounds having straight alkyl chains attached to polar groups (8,15). This complex pattern obtained for ΔH_{soln}^0 of homologous alkyl compounds as a function of carbon atoms reflects mainly the differences in the interactions between solute molecules in the pure state. This can be seen from figure 3, where the enthalpy of solvation, ΔH_{solv} , is plotted against the number of carbon atoms. Where no solute-solute interactions occur, a more uniform picture emerges. The ΔH_{solv} increment for the CH_2 group appears to be reasonably constant after $n=4$. The increment value, $-3.6 \text{ kJ}\cdot\text{mol}^{-1}$, is similar to that found for RNH_2 , ROH , and RCOOH (8).

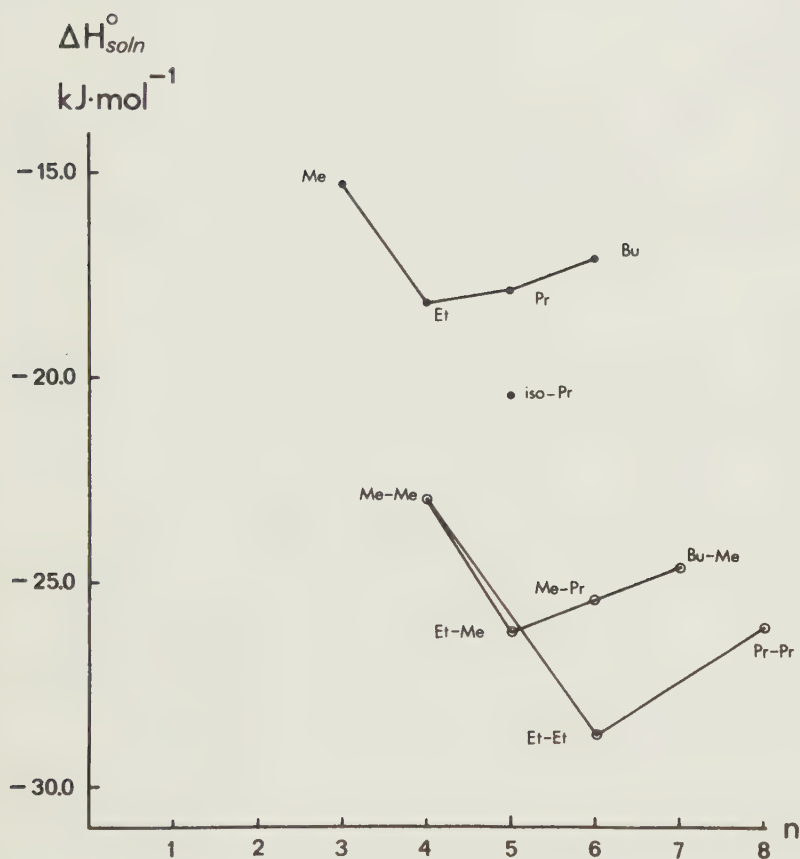
It is seen that the large difference between ΔH_{soln} values for straight chain and branched propyl is only very weakly reflected in the ΔH_{solv} values. The same effect was found in ref 8. It is thus evident that the ΔH_{solv} group value for CH_2 is not very sensitive to the structure of the alkyl chain or to the functional group, at least for n larger than 4.

The ΔC_p^0 values for a solution process reflect the properties of the pure compound as well as that for the solution. Values of $\bar{C}_{p,2}^0$ are therefore better suited for discussion of solute-solvent interactions, as the $\bar{C}_{p,2}^0$ value is not affected by the solute-solute interaction. In figure 4 values of $\bar{C}_{p,2}^0$ are plotted against n and it is seen that the values for the two series of compounds form two nearly identical

Table 2. Thermodynamic data for some alkoxyethanols and dialkoxyethanes at 25°C

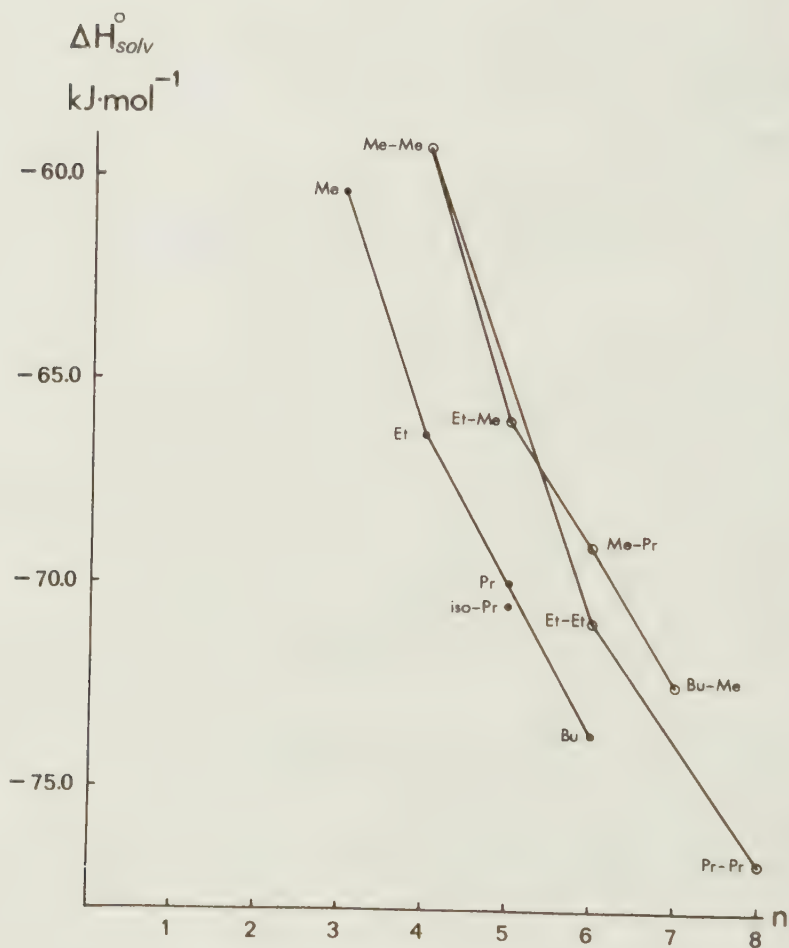
Compound	$-\Delta H_{\text{soln}}$ $\text{kJ}\cdot\text{mol}^{-1}$	$-\Delta H_{\text{soln}}$ $\text{kJ}\cdot\text{mol}^{-1}$	$\Delta C_{p,2}^0$ $\text{kJ}\cdot\text{mol}^{-1}$	$\bar{C}_{p,2}^0$ $\text{kJ}\cdot\text{mol}^{-1}$	C_p^0 $\text{kJ}\cdot\text{mol}^{-1}$
$\text{MeOCH}_2\text{CH}_2\text{OH}$	15.27 ± 0.02	60.44 ± 0.04	115 ± 4	290 ± 4	174.9 ± 0.2
$\text{EtOCH}_2\text{CH}_2\text{OH}$	18.15 ± 0.03	66.36 ± 0.06	176 ± 5	387 ± 5	210.8 ± 0.2
$\text{PrOCH}_2\text{CH}_2\text{OH}$	17.83 ± 0.02	69.95 ± 0.10	226 ± 5	468 ± 5	241.6 ± 0.2
$i\text{-PrOCH}_2\text{CH}_2\text{OH}$	20.41 ± 0.01	70.53 ± 0.09	218 ± 6	457 ± 6	238.8 ± 0.3
$\text{BuOCH}_2\text{CH}_2\text{OH}$	17.05 ± 0.03	73.64 ± 0.04	283 ± 7	556 ± 7	273.1 ± 0.1
$\text{MeOCH}_2\text{CH}_2\text{OMe}$	22.95 ± 0.03	59.34 ± 0.04	187 ± 7	380 ± 7	193.3 ± 0.5
$\text{EtOCH}_2\text{CH}_2\text{OMe}$	26.21 ± 0.05	66.04 ± 0.06	238 ± 7	463 ± 7	224.7 ± 0.2
$\text{EtOCH}_2\text{CH}_2\text{OEt}$	28.72 ± 0.04	71.92 ± 0.06	310 ± 7	569 ± 7	259.4 ± 0.2
$\text{PrOCH}_2\text{CH}_2\text{OMe}$	25.44 ± 0.03	69.09 ± 0.04	314 ± 7	563 ± 7	248.9 ± 0.2
$\text{BuOCH}_2\text{CH}_2\text{OMe}$	24.65 ± 0.03	72.48 ± 0.06	370 ± 10	652 ± 10	282.0 ± 0.2
$\text{PrOCH}_2\text{CH}_2\text{OPr}$	26.17 ± 0.08	76.79 ± 0.16	392 ± 10	701 ± 10	309.0 ± 0.3

Figure 2. Enthalpies of solution at infinite dilution at 25°C plotted against the number n of carbon atoms.



$\bullet$, $\text{ROCH}_2\text{CH}_2\text{OH}$; $\circ$, $\text{ROCH}_2\text{CH}_2\text{OR}'$

Figure 3. Enthalpies of solvation at 25°C plotted against the number n of carbon atoms.



●, $\text{ROCH}_2\text{CH}_2\text{OH}$; ○, $\text{ROCH}_2\text{CH}_2\text{OR}'$

straight lines. The slope of the lines is practically identical with that found by Konicek and Wadsö (8) for other alkyl compounds, cf the dashed line in figure 4. The $\bar{C}_{p,2}^0$ values for the present series of compounds and for those investigated earlier (8) can be expressed by a linear equation

$$\bar{C}_{p,2}^0 = a + b \cdot n \quad (3)$$

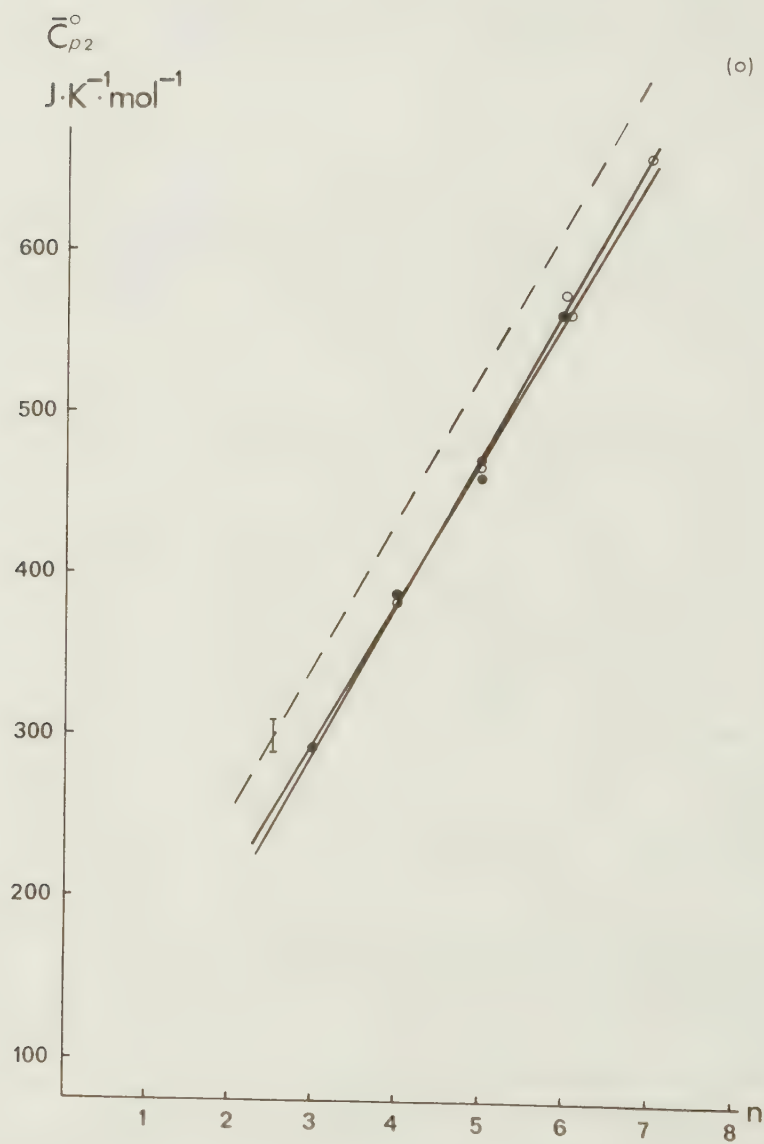
where the slope b is equal to the increment in $\bar{C}_{p,2}^0$, accompanying the addition of a CH_2 group. In table 3 values for the constants a and b are listed. They were obtained by least square treatment for the series of non-ionic compounds, both straight and branched chain, investigated so far.

Table 3. Constants derived for the equation $\bar{C}_{p,2}^0 = a + b \cdot n$.

	$a \pm 25$	$b \pm 25$
	$\text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$	$\text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$
Alcohols	64 ± 8	96 ± 4
Amines	58 ± 12	90 ± 2
N-substituted amides	86 ± 12	86 ± 2
Carboxylic acids	87 ± 4	84 ± 2
Alkoxyethanols	32 ± 12	87 ± 2
1,2-Dialkoxyethanes	9 ± 14	92 ± 2

Inspection of table 3 shows that the values of b are nearly identical for all series of compounds. The conclusion is that the "hydrophobic effect", as reflected by $\bar{C}_{p,2}^0$, is strongly correlated to the number of alkyl carbon atoms, but it is not very sensitive to the structure of the alkyl carbon chain or to the nature of the functional groups attached to it. This is the same conclusion as obtained for the ΔH_{solv} increment, but the heat capacity effect is much more pronounced and uniform.

Figure 4. Partial molar heat capacities $\bar{C}_{p,2}^0$ at 25°C plotted against the number n of carbon atoms.



●, $\text{ROCH}_2\text{CH}_2\text{OH}$; ○, $\text{ROCH}_2\text{CH}_2\text{OR}'$. The dashed line represents the best fit for the values of $\bar{C}_{p,2}^0$ found for RCOOH , RNH_2 , ROH , and RCONHR' .

SPECIFIC HEAT MEASUREMENTS ON PROTEINS (Paper IV)

The specific heat measurements with the drop heat capacity calorimeter II were performed on ovalbumin, chymotrypsinogen and lysozyme in the solid state at different water contents and in aqueous solution at different concentrations at 25°C. The partial specific heats were derived from the measured specific heat, c_p , by use of eqn 4 (cf ref 17)

$$(1+w_2)c_p = \bar{c}_{p,1} + w_2 \cdot \bar{c}_{p,2} \quad (4)$$

where $\bar{c}_{p,1}$ and $\bar{c}_{p,2}$ are the partial specific heats for the solvent and solute, respectively, and w_2 is the weight quotient between the masses of solute and solvent. For the solids the protein component is considered as the solvent. The plot of $(1+w_2)c_p$ against w_2 gives $\bar{c}_{p,1}$ as the ordinate intercept and the $\bar{c}_{p,2}$ as the slope if the curve is linear in the range studied. Measurements on solid proteins were made in the range of 1-18 per cent water content. From figure 5, showing the results for lysozyme, it is seen that a small negative deviation from a straight line was obtained in the middle range. Similar patterns were found for chymotrypsinogen and ovalbumin. This effect is believed to be due to a lower partial specific heat of water in this range. From the aqueous solution experiments straight lines were obtained up to protein concentrations of 6-10 weight per cent.

The results from the experiments on solid proteins together with values found in the literature are summarized in table 4. The poor agreement between the protein values from this study and from the literature is probably largely due to the definition of the dry protein state. In the present study the dry protein was taken to be the state derived after 24 hours drying in an oven at atmospheric pressure and 105°C.

The comparison of the specific heat values of lysozyme and chymotrypsinogen with the specific heat of their component amino acids indicates that it is possible to calculate specific heats for proteins from their amino acid composition within a few per cent. From the derived eqn 5 the specific heat, c_p^0 , for a protein with n amino acids, molecular

Figure 5. Results of specific heat measurements on solid lysozyme as a function of the water content.

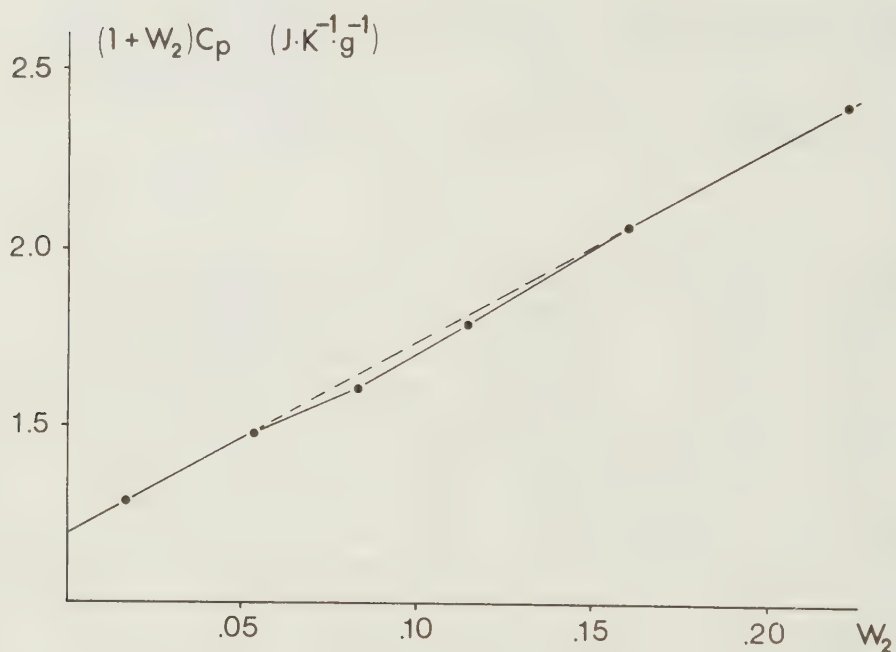


Table 4. Partial specific heats at zero water contents of solid lysozyme, chymotrypsinogen and ovalbumin at 25°C.

	This study		Lit.
	Protein $\bar{c}_{p,1}^0/\text{J}\cdot\text{K}^{-1}\cdot\text{g}^{-1}$	Water $\bar{c}_{p,2}^0/\text{J}\cdot\text{K}^{-1}\cdot\text{g}^{-1}$	Protein $\bar{c}_{p,1}^0/\text{J}\cdot\text{K}^{-1}\cdot\text{g}^{-1}$
Lysozyme	1.192 ± 0.005	5.42 ± 0.03	
Chymotrypsinogen	1.223 ± 0.004	5.46 ± 0.03	1.293 ± 0.003^a
Ovalbumin	1.231 ± 0.005	5.53 ± 0.04	1.97 ± 0.08^b 1.18 ± 0.52^c

^aRef 18

^bRef 19

^cRef 17

weight M and i peptide chains can be calculated (20,21)

$$\bar{c}_p^0 = \frac{\sum C_p(\text{amino acid}) - (n-i)34.7}{M} \quad (5)$$

$\sum C_p(\text{amino acid})$ is the sum of the heat capacities of the amino acids, and $(n-i)34.7$ ($\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$) is a correction for the formation of $(n-i)$ peptide bonds. From the fact that the specific heat values for amino acids (21) differ only slightly from each other (with the exception of methionine and valine) and from the derived eqn 5, it was concluded that $\bar{c}_{p,1}^0$ values for most proteins can be expected to be close to $1.2 \text{ J}\cdot\text{K}^{-1}\cdot\text{g}^{-1}$.

The table 5 summarizes the results from aqueous solution experiments.

Table 5. Partial specific heats of infinite aqueous dilution of lysozyme, chymotrypsinogen and ovalbumin at 25°C

	This study Water $\bar{c}_{p,1}^0/\text{J}\cdot\text{K}^{-1}\cdot\text{g}^{-1}$	Protein $\bar{c}_{p,2}^0/\text{J}\cdot\text{K}^{-1}\cdot\text{g}^{-1}$	Lit. Protein
Lysozyme	4.1792 ± 0.0004	1.494 ± 0.007	1.30 ± 0.04^a
Chymotrypsinogen	4.1808 ± 0.0008	1.529 ± 0.015	1.60 ± 0.05^b
Ovalbumin	4.1778 ± 0.0004	1.534 ± 0.014	1.91 ± 0.05^c 1.66 ± 0.09^d

^aRef 22

^bRef 23

^cRef 19

^dRef 17

The most striking result from the $\bar{c}_p$ measurements is the large and nearly identical $\Delta\bar{c}_p$ value obtained for the transfer of the proteins from their solid state to aqueous solution. The $\Delta\bar{c}_p^0(\text{solv})$ values for the proteins are 0.302 ± 0.008 , 0.306 ± 0.015 and 0.308 ± 0.015 $\text{J}\cdot\text{K}^{-1}\cdot\text{g}^{-1}$ for lysozyme, chymotrypsinogen and ovalbumin, respectively.

An attempt was made to analyse the obtained $\bar{c}_{p,2}^0$ values by use of model compound data and simple additivity rules. The different contributions to the $\bar{c}_{p,2}^0$ values were separated into several groups: A. back bone, B. polar groups, C. non-polar groups and D. ionisation. The group B consists of two $\bar{c}_p$ effects: the $\bar{c}_p^0$ values for non-solvated, non-polar groups and a contribution accounting for the transfer of the groups to aqueous solution, $\alpha\Delta\bar{c}_p$, where α is the value for the degree of solvation of the non-polar groups ($\alpha=1$, at complete solvation). The estimated contributions from the different groups are summarized in table 6.

The $\bar{c}_{p,2}^0$ can be written as the sum of all the different contributions

$$\bar{c}_{p,2}^0 = \bar{c}_p(\text{back bone}) + \bar{c}_{p,2}^0(\text{polar}) + \bar{c}_p^0(\text{non-polar}) + \alpha\Delta\bar{c}_p(\text{solv}) + \Delta\bar{c}_p(\text{ion}) \quad (6)$$

Table 6. Contributions from the different groups to the total heat capacity of a protein in aqueous solution

A. <u>Contributions from the back bone</u>		$C_p^O(\text{back bone}) = n \cdot 99.20 - (n-1) \cdot 33.80$ (J.K ⁻¹ ·mol ⁻¹)		Eqn 5	
		^{34.7} 33.80			
B. <u>Contributions from solvated polar groups</u>		$\bar{C}_{p,2}^O(\text{polar})$			
Alcohol	(phenol.)	$\bar{C}_{p,2}^O(\text{polar}) =$	64 (J.K ⁻¹ ·mol ⁻¹)	Ref 8	
"			-30	27*	
Amino			58	8	
Carboxylic			87	"	
Amido			86	"	
Guanidino			140	Estimated	
Imidazole			110	"	
C. <u>Contributions from non-polar groups, $\bar{C}_{p,2}^O = C_p^O(\text{non-polar}) + \alpha \Delta C_p(\text{solv})$</u>		$C_p^O(\text{non-polar}) =$		Ref 21*	
Non cyclic aliphatic (CH ₂ increment)		23.5 (J.K ⁻¹ ·mol ⁻¹)			
Cyclic aliphatic	(" ")	17.3			
Aromatic	(-CH=, ")	13.5			
Benzene		80.8			
Indole		115.9			
Monosulfide		114.3**			
Disulfide		17.4**			
				Cont.	

Table 6. Cont.

Non cyclic aliphatic (CH ₂ increment)		
Cyclic aliphatic (" ")	$\Delta C_p(\text{soln}) = \frac{65.7}{59.2}$	(J·K ⁻¹ ·mol ⁻¹) Ref 8,21*)
Aromatic (-CH=, " ")	54.5	Estimated
	43.2	Ref 8,21,27*)
D. <u>Contribution from ionisation</u> $\Delta C_p(\text{ion})$		
	$\Delta C_p(\text{ion}) = -167$	(J·K ⁻¹ ·mol ⁻¹) Ref 28

*) Calculated from values given in the references.

**) Simple additivity rules do not seem to apply for the mono- and disulfide groups. However the errors for these values are compensated in the final calculation.

In order to fit the experimental results with data calculated from eqn 6 (cf table 7) α values of 0.27 and 0.20 were found for lysozyme and chymotrypsinogen, respectively. The α values thus obtained agree well with the values given in ref 24, 25 and 26 for the degree of solvation of non-polar groups.

THE AVIDIN-BIOTIN REACTION (Paper V)

The enthalpy measurements for the binding of biotin to avidin were performed on an LKB-10700-2 batch microcalorimeter at 25, 34 and 43 °C. (pH 9, ammonium acetate buffer). At 25°C a calorimetric titration curve for the avidin-biotin reaction was constructed by varying the avidin concentration. The obtained curve is typical for the formation of a strong complex. The equivalence point derived corresponded to 10.5 ± 0.1 μ g biotin/mg avidin, which when compared with the value determined by Green *et al.* (29) suggested the presence of about 30 % non-active material. However, this low purity should not significantly interfere with the result because of the high specificity of the binding reaction.

The results from heat of reaction measurements are $\Delta H(25^\circ\text{C}) = -94.1 \pm 0.4$ kJ/mol biotin, which is the mean enthalpy of binding of all four binding sites of avidin. From the enthalpy values $\Delta C_p = -0.99 \pm 0.05$ kJ/K·mol biotin was derived.

Spectroscopic studies of the avidin-biotin reaction (30) led Green to suggest large contributions from hydrophobic interactions to the free energy of binding. This is in agreement with the highly negative ΔC_p value derived here. The ΔC_p value estimated from model compound experiments (8) for the transfer of the hydrophobic part of the biotin molecule from water to a non-aqueous environment is -565 J/K·mol biotin, which is about 400 J/K·mol too small, compared with the experimentally obtained value. This difference could be accounted for if the assumed hydrophobic binding site prior to the reaction has contact with the water, and upon binding, this water is excluded with a substantial decrease in heat capacity.

Table 7. Total contributions from the different groups to the heat capacity of lysozyme and chymotrypsinogen.

	$\bar{C}_{p,2}^0 \text{ (J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1} \text{)}$	<u>Lysozyme</u>	<u>Chymotrypsinogen</u>	$\text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$
Observed		<u>21 385</u>	<u>39 243</u>	
Calculated contributions from				
Back bone, C_p^0 (back bone)		8 354	15 835	"
Polar groups, $\bar{C}_{p,2}^0$ (polar)		5 289	8 040	"
Non-polar groups, C_p^0 (non-polar)		6 927	12 867	"
Solvation of non-polar groups, ΔC_p (solv)		18 669	+35 613	"
Ionisation, ΔC_p (ion)		-4 175	-4 509	"
Complete solvated $\bar{C}_{p,2}^0$ (solv)($\alpha=1$)		<u>35 064</u>	<u>67 846</u>	"
The degree of solvation, α		0.27	0.20	

ACKNOWLEDGEMENTS

I wish to express my sincere thanks and appreciation to Dr. Ingemar Wadsö for his comprehensive support, encouragement and many helpful suggestions throughout the course of this work.

I am very grateful to Mr. Sven Hägg for the design and building of a substantial part of the electronic equipments used, and to Mr. Börje Pettersson and Mr. Lars Palm at the mechanical workshop of the Chemical Center for their cooperation throughout this work. I am also indebted to Professor Stig Sunner and Dr. Margret Månsson for many helpful discussions and to Mrs. Gerd Hornemark for her skilful typing of all the manuscripts. To all friends and colleagues at the Thermochemistry Laboratory, including a large number of foreign guest workers, who have helped in many different ways I extend my warmest thanks.

Dr. Peter Sellers, Professor Charles H. Spink and Dr. Norman Nichols revised the English of the various manuscripts. To all of them I want to express my sincere gratitude.

Financial support from the Swedish Natural Science Research Council, the Swedish Board for Technical Development and the Faculty of Mathematics and Natural Science, University of Lund, is gratefully acknowledged.

Finally, I wish to thank my wife, Mona, for her patience, encouragement and help, without which this work would not have been completed.

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